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Life Histories and Senescence of *Botryllus schlosseri* (Chordata, Ascidiacea) in Monterey Bay

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Abstract. The colonial ascidian *Botryllus schlosseri* is a model organism for research on invertebrate histocompatibility, development, and evolutionary biology. Nonetheless, the basic life history of Pacific Ocean populations of the species remains unknown. We determined field rates of growth, reproduction, and senescence in four cohorts of *B. schlosseri* colonies in Monterey Bay, California. Colonies grew exponentially as juveniles and reached sizes of up to 1400 zooids within 69 days. After a juvenile phase lasting at least 49 days, the colonies began to reproduce sexually. Each zooid produced up to 10 clutches, each with a maximum of 5 eggs, resulting in very high fecundity of up to 8000 eggs per colony. Following a short period (maximum 70 days) of continuous sexual reproduction, colonies abruptly senesced and died while still bearing a full clutch of eggs. Senescence progressed through four distinct stages over 1–2 weeks, and inevitably led to the simultaneous death of all zooids in the colony. Although senescence was the main cause of mortality, some colonies died as a result of predation or undetermined causes. Certain life history traits varied significantly between cohorts that settled at different times of year. For example, lifespans in the field varied from about 3 months for spring to 8 months for fall-born colonies, but the lifetime fecundity of colonies did not vary between cohorts. The morphologies and life histories of colonies monitored in the field and reported here differed from those of colonies cultured previously in the laboratory.

Introduction

The colonial ascidian *Botryllus schlosseri* Pallas is widely employed in studies on invertebrate alloimmunity (reviewed by Weissman *et al.*, 1990; Rinkevich, 1992; Sabbadin *et al.* 1992), development (Milkman, 1967; Mukai and Watanabe, 1976; Rinkevich *et al.*, 1992; Lauzon *et al.*, 1993), and evolutionary ecology (Grosberg, 1988; Harvell and Grosberg, 1988; Buss, 1990). Most of this research has been conducted under laboratory conditions. Life histories of *B. schlosseri* colonies in the field are known for some populations in the Atlantic Ocean (Grave, 1933; Grosberg, 1988) and Mediterranean Sea (Brunetti, 1974). The life histories of Pacific Ocean populations are, however, little known, despite their extensive use in laboratory investigations (Scofield *et al.*, 1982; Rinkevich and Weissman, 1987; Lauzon *et al.*, 1933, and references therein) (Carwile, 1989). *B. schlosseri* was probably introduced to the Pacific Ocean sometime during the last century as one of the fouling organisms on wooden-hulled vessels or concomitant with the culture of Atlantic oysters, which transferred whole organisms and their encrusting communities from the Atlantic to the Pacific Ocean (Carlton, 1987; Hewitt, 1993). Recent morphological and genetic studies indicate that *B. schlosseri* at Woods Hole (Atlantic Ocean) and Monterey (Pacific Ocean) are the same species (Boyd *et al.*, 1990). Results of laboratory cultures from both of the latter populations have been used to infer evolutionary processes in nature (Harvell and Grosberg, 1988; Weissman *et al.*, 1990; Rinkevich, 1992, and references therein). Thus, it becomes important to understand the life history patterns of *B. schlosseri* from different populations and under different culture conditions.

We present here the life histories of *B. schlosseri* colonies growing under field conditions in Monterey Bay, California. We describe patterns of growth, sexual reproduction, and senescence in cohorts that settled at four times of year. We then compare these field life histories

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with those of laboratory-cultured colonies from the same population.

Materials and Methods

We conducted the present study during 1990–1991 in the Monterey Municipal Marina, Monterey County, California (36° 37.4'N, 121° 54'W). Surface seawater temperatures varied from 11.4°C in January to 16.5°C in August (E. C. Haderlie, pers. comm.). This site is described in detail by Haderlie and Donat (1978) and Carwile (1989). Colonies of *Botryllus schlosseri* grow on docks, floats, and pilings throughout the marina and seasonally dominate the fouling community (pers. obs., N. E. Chadwick-Furman). Colonies of *B. schlosseri* sexually reproduce throughout the year at Monterey; sexual generations overlap and cohorts are not discrete (Carwile, 1989).

To determine life history patterns, we monitored cohorts of *B. schlosseri* that settled at four arbitrarily chosen dates: 19 May 1990, 3 July 1990, 15 October 1990, and 25 January 1991. To obtain each cohort, we collected 10 large colonies from wooden pilings at 0–1 m depth in the marina, transported them to Hopkins Marine Station of Stanford University, and maintained them in flowing seawater at ambient temperature. We secured the colonies with string to glass plates and placed them vertically in aquaria, with an empty plate facing each colony. Within a few days, they released swimming larvae that rapidly settled and metamorphosed into sessile zooids on the facing plates. We then isolated each newly settled zooid on a separate 5.0 × 7.5 cm glass plate, and allowed it to firmly attach during 1 week in the laboratory. For each cohort, we transplanted at least 25 newly settled, isolated zooids to the marina field site.

In the marina, we placed the zooids in wooden racks and hung them face down from floating docks at 0.5–1.0 m depth (after Brunetti, 1974; Boyd *et al.*, 1986; Grosberg, 1988). Sessile organisms colonized the racks and formed a fouling community around the experimental plates (see Carwile, 1989, for community description). No epibionts were observed to settle on individuals of *B. schlosseri*.

At each sample interval, every 4–7 days, we observed the growing colonies in the laboratory and then returned them to the field within a few hours (for details of methods, see Brunetti, 1974; Grosberg, 1988). They showed no adverse effects of handling (see also Milkman, 1967). To avoid effects of crowding on colony growth, we removed all other organisms from the plates during each sample interval (after Brunetti and Copello, 1978; Grosberg, 1988). Colonies grew over both sides of the plates, but did not fill all of the space provided. About every 7 days, depending on the time of year, all the zooids in each colony passed through an asexual growth cycle (hereafter termed "cycle"). During each cycle, the zooids produced buds, then shrank and were replaced by their buds, which formed a new asexual generation of zooids in each colony. The replacement of zooids during each cycle in *Botryllus* is described in detail by Mukai and Watanabe (1976) and Grosberg (1988). Here we report colony age in terms of both the number of cycles and the days since settlement (after Brunetti and Copello, 1978; Grosberg, 1988).

During each sample interval, we determined the number of zooids, number of eggs, cycle stage (see Mukai and Watanabe, 1976), and the general condition of each colony. In colonies of less than 800 zooids, we counted zooid number directly. For larger colonies, zooid number was estimated by placing a grid over the colony surface, counting all zooids under a single grid-square (1.5 × 1.8 cm), and multiplying by the number of squares occupied by the entire colony (maximum = 12). To determine the number of eggs per colony, we visually estimated the number of eggs per zooid, then multiplied by the total number of zooids. Eggs were observed from the exterior of whole, undissected colonies.

The causes of mortality were determined by analyzing colony morphology. In senescing colonies, the entire organism deteriorated in distinct stages during the 1–2 weeks preceding death (Brunetti, 1974; Rinkevich *et al.*, 1992). Colonies that senesced left behind a residue of decaying tissue that distinguished them from colonies killed by other agents. In cases of predation, colonies showed localized lesions and then sections of dead tissue that increased in area for several weeks before the complete consumption of the colony by predators.

Results

Morphology and growth

In the Monterey Marina, members of all cohorts of *Botryllus schlosseri* exhibited the same general morphology. Colonies were flat and roughly circular to oval in outline. Their zooids were closely packed, with almost no space between adjacent zooids or systems (circular groups of zooids). Each colony formed a compact disk that did not fragment.

Juvenile colonies in all cohorts grew exponentially (Fig. 1). Colonies that settled in May grew significantly faster than those in all other cohorts (Fig. 2A, Table I) and reached a size of up to 1400 zooids in 69 days. Members of the July and October cohorts grew the slowest, at rates that did not differ significantly (Fig. 2a, Table I). Colonies that settled during October and January delayed their exponential growth until the spring months (Fig. 1). Some colonies reduced their growth rates after commencing sexual reproduction (Fig. 1). In addition, 15.6% of all colonies (N = 122) shrank slightly (by 15.2% + 11.1% in zooid number, x + SD) during the 2 weeks preceding

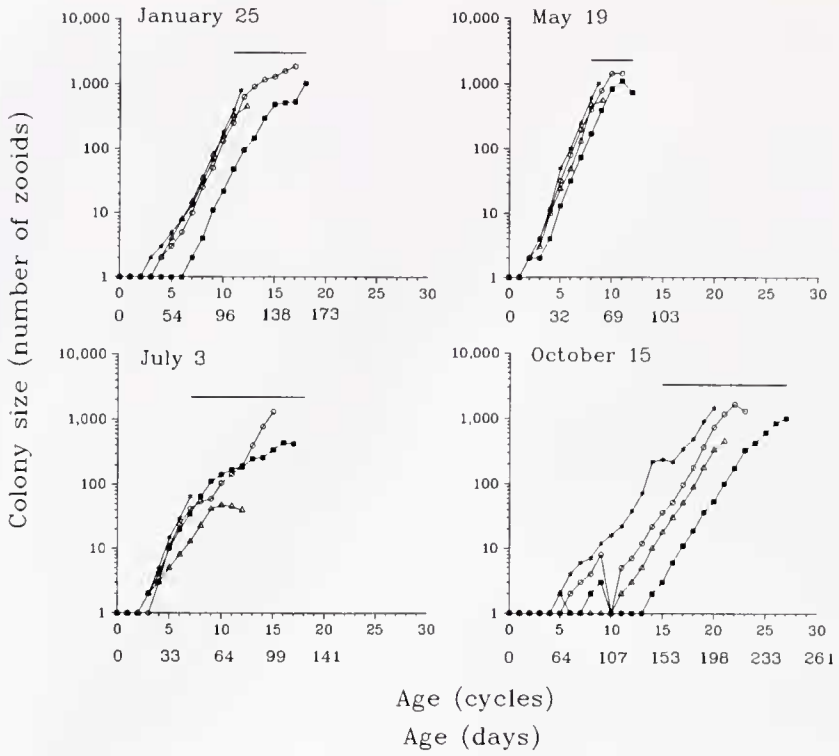

Figure 1. Growth, reproduction and lifespan in four cohorts of the colonial ascidian *Botryllus schlosseri* in Monterey Bay. For clarity, growth curves are presented for only four colonies per settlement date (out of >25 original); they represent the extremes of those that survived to reproduce in each cohort: the smallest in final size (triangle), largest in final size (circle), shortest-lived (asterisk), and longest-lived (square). Horizontal bars indicate the period of sexual reproduction for the entire cohort. Note that colony size is plotted on a logarithmic scale.

death. The only other lapse in exponential growth occurred when members of the October cohort were attacked by an unknown predator during cycles 5–15 (Fig. 1).

Sexual reproduction

After a period of exponential somatic growth, the colonies entered sexual reproduction. The May and July cohorts reached sexual maturity the earliest, at ages that did not differ significantly (Fig. 2b, Table I). The minimum age at sexual maturity was 49 days (7 cycles). Members of the October cohort began to reproduce when significantly older than other cohorts (Table I, Fig. 2b). They overwintered as small juveniles and postponed reproduction until spring, at a minimum age of 153 days (15 cycles) (Fig. 1).

The size at which colonies began to reproduce varied widely (range = 38 to 1297 zooids) but did not differ significantly between most cohorts (Table I). Colonies that settled in July reproduced at the smallest sizes; those in January and May at the largest (Fig. 2c). Due to differences in growth rate, some cohorts matured at similar sizes but widely different ages (compare May and July cohorts, Fig. 2b and c).

Most zooids in each colony produced eggs continuously throughout the period of sexual reproduction. The duration of reproduction extended for 7–70 days (1–10 cycles), with one clutch of eggs produced during each cycle. Some cohorts produced significantly more clutches than did others (Table I). Each zooid contained up to five eggs per cycle, although most zooids produced only one to two eggs per cycle (Fig. 2d). Colonies did not interrupt clutch production or reduce the number of eggs per zooid as they aged; mature colonies still contained a full clutch of eggs when they died.

Lifetime fecundity was very high (maximum = about 8000 eggs). Such high fecundity was possible because of the large number of zooids in adult colonies and their ability to produce multiple clutches. Lifetime fecundity did not differ significantly between colonies that settled at different times of year (Table I, Fig. 2f).

Longevity and survivorship

Colonies grown in the field at Monterey had short, subannual lifespans. Maximum lifespan ranged from almost 3 months (82 days) in the May cohort, to just over 8 months (247 days) in the October cohort (Fig. 3).

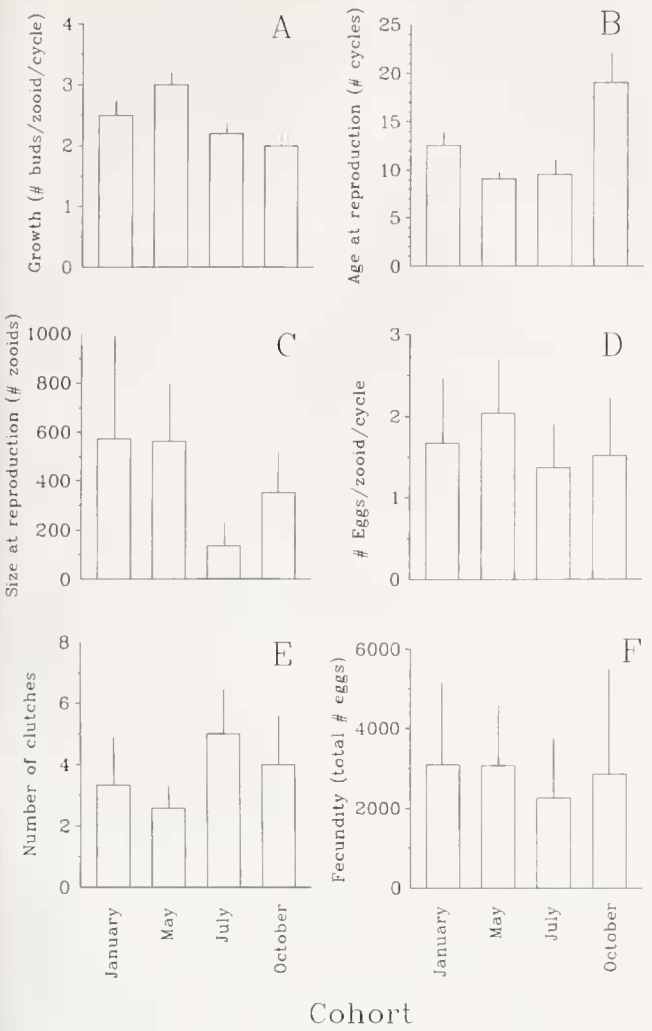

Figure 2. Variation in six life-history traits among four cohorts of the colonial ascidian *Botryllus schlosseri* in Monterey Bay. Error bars: positive standard deviations of the means.

The percentage of colonies that survived to first reproduction was high in most cohorts (Fig. 3). Colonies that settled in October had lower survivorship to maturity than did the other three cohorts. An undetermined predator began to attack members of the October cohort during cycle 5. Mortality increased gradually, and by the 15th cycle, only 21% of the cohort remained (Fig. 3). Nevertheless, the remaining October colonies all eventually reproduced sexually and lived longer than those of any other cohort (Figs. 1 and 3). In all cohorts, survivorship decreased rapidly after commencement of sexual reproduction, and all colonies then died within 10 cycles (Fig. 3).

Senescence caused most of the mortality in field-raised colonies (54.9% of colonies, $N = 122$). After a period of continuous sexual reproduction, colonies passed through four stages of degeneration, as previously described for *B. schlosseri* (Brunetti, 1974; Rinkevich *et al.*, 1992). First, blood vessels narrowed and blood flow slowed. Then, the zooids shrank and became densely pigmented. In the third stage, circular systems (groups) of zooids were disconnected and became disorganized. In the fourth and final stage, the protective tunic softened and disintegrated, and all of the tissue died. A film of tunic material persisted for at least 1 week after death, and marked the former extent of the colony. Senescence was not reversible. In all cases, the initial stages of senescence led to the death of the entire colony within 1–2 weeks. Some colonies senesced while still in the juvenile stage, at an age of at least 70 days (= 10 cycles), and died without reproducing sexually. The occurrence and timing of senescence did not appear to be related to the position of the colonies in the racks or to other extrinsic factors.

Other agents of mortality included predation (12.3% of colonies, $N = 122$, described above), and undetermined causes of death early in life (32.8%, $N = 122$). In the latter case, small juvenile colonies suddenly disappeared from the field site without showing any previous signs of damage.

Discussion

We demonstrate here that *Botryllus schlosseri* colonies raised in the field at Monterey have characteristic morphologies, which are readily distinguishable from those of colonies grown under laboratory conditions. In the field, isolated colonies are rounded and compact (Brunetti, 1974; Grosberg, 1988; Carwile, 1989; this paper). In con-

Table I

Tukey-Kramer multiple comparisons test for differences in life history traits between cohorts of the ascidian Botryllus schlosseri *grown in Monterey Bay, California, during 1990–1991*

Life-history trait	Cohort*			
Growth rate (#buds/zooid/cycle)	Jan	May	Jul	Oct
Age at 1st reprod. (# cycles)	Jan	May	Jul	Oct
Size at 1st reprod. (# zooids)	May	Jan	Oct	Jul
Number of eggs/zooid/clutch	May	Jan	Oct	Jul
Clutch number	Jan	May	Oct	Jul
Fecundity (total # eggs/colony)	Jan	May	Jul	Oct

* Cohorts that did not differ significantly ($p > 0.05$) are conjointly underlined. See text for details.

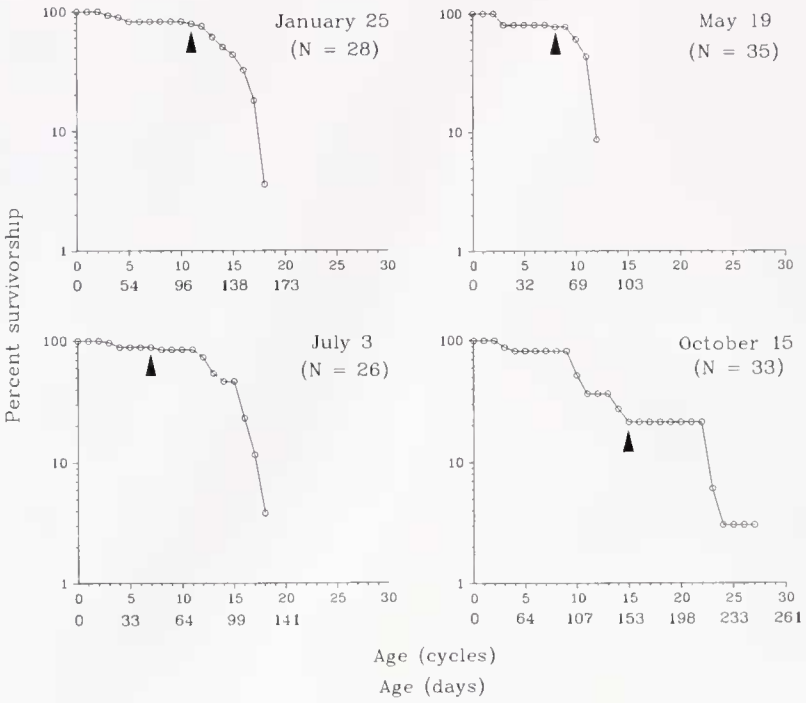

Figure 3. Survivorship curves of the colonial ascidian *Botryllus schlosseri* in Monterey Bay. Presented are four cohorts, each settled at the date shown. Arrows indicate the beginning of sexual reproduction in each cohort. Note that survivorship is plotted on a logarithmic scale.

trast, colonies grown in the laboratory tend to branch and produce extensions along the substratum; these extensions may fragment into subcolonies (Rinkevich and Weissman, 1987; N. E. Chadwick-Furman, pers. obs.).

In addition, the life history patterns of colonies in the field seem to differ from those in the laboratory. Members of all field cohorts at Monterey exhibited the same general features: rapid growth, short and intense reproduction, short lifespan, and senescence soon after reaching maturity (Figs. 1–3). In contrast, Monterey colonies raised in the laboratory have been observed to grow slowly or shrink over many months, to cease reproduction long before death, and to live for more than 2 years (Boyd *et al.*, 1986; Rinkevich and Weissman, 1987; Rinkevich *et al.*, 1992). Also in Mediterranean populations, the same life history differences are exhibited between field- and laboratory-raised colonies (Brunetti, 1974; Brunetti and Copello, 1978).

Several factors may be responsible for these differences. Under laboratory conditions, water motion is slower, and particulate food is less varied and abundant than for *B. schlosseri* populations in the field (Milkman, 1967; Brunetti and Copello, 1978; Carwile, 1989). In addition, the absence of natural grazers in the laboratory may lead to the formation of a fouling film that inhibits the attachment and growth of colonies (Boyd *et al.*, 1986; N. E. Chadwick-Furman, pers. obs.). Laboratory culture is important for the maintenance of genetically defined stocks that are employed in histocompatibility studies and other investigations (reviewed in Sabbadin *et al.*, 1992; Rinkevich *et al.*, 1992). Laboratory culture at summer temperatures also allows continued production of experimental tissues during the winter when field colonies in Monterey slow their growth (Boyd *et al.*, 1986). Unfortunately, however, the life history traits exhibited by laboratory cultures, including patterns of growth, reproduction, and longevity, may not reflect the evolutionary or ecological processes that act upon *B. schlosseri* in nature.

In the field at Monterey, life history variation between cohorts is probably related to seasonal environmental cycles. Factors known to correlate with such variation in other field populations of *B. schlosseri* include water temperature and particulate food concentration (Millar, 1971; Brunetti, 1974). In the present study, we observed slowed growth and delayed reproduction of young colonies during the winter months when temperature and planktonic food levels are at their annual minima in Monterey Bay (Haderlie and Donat, 1978; Boyd *et al.*, 1986; Carwile, 1989, and references therein). The colonies began to grow exponentially and reproduce sexually in the spring to summer when the above two factors reach their annual maxima. In some localities, *B. schlosseri* colonies completely cease sexual reproduction during the winter when temperatures fall below 11°C (Millar, 1971). At Monterey,

however, sea temperature remains above 11°C all year, so mature colonies continue to produce eggs even during January (see Methods).

The present study had several weaknesses. Although colonies were cultured in the sea, they did not grow under completely natural conditions. The periodic removal of competitors may have led to inflated rates of growth and reproduction. Thus, values reported here are probably maximal in the absence of space competition. Also, we did not monitor colonies immediately following natural settlement in the field. Thus, survival rates are probably inflated because we did not determine natural mortality rates during the first 1–2 weeks of life. Finally, we conducted our study in an artificial habitat, on a non-native population of *B. schlosseri*. As such, the life histories presented here are not those of a natural field population. Members of this species probably have been introduced over much of their current range, possibly from native populations in the Mediterranean Sea (Carlton, 1987; Hewitt, 1993). Indeed, a weakness of most life history studies on this species is that they have been conducted on introduced populations or in manmade fouling environments, or both (Millar, 1971; Brunetti, 1974; Grosberg, 1988; Carwile, 1989).

In spite of the above drawbacks, the data presented here give the most complete picture to date of life histories and morphologies of Pacific Ocean populations of *B. schlosseri*. The life histories of Monterey Bay colonies are quite similar to those of iteroparous colonies at Woods Hole in the western Atlantic (Grave, 1933; Grosberg, 1988), and at the Venice Lagoon in the Mediterranean (Brunetti, 1974).

We present here the first description of senescence in the field for Monterey *B. schlosseri*. Our observations confirm that senescence progresses under field conditions through essentially the same stages as in the laboratory, and takes about 1–2 weeks (Brunetti and Copello, 1978; Rinkevich *et al.*, 1992). Senescence appears to be controlled intrinsically, as inferred from the synchronized death of segregated laboratory clones (Rinkevich *et al.*, 1992). The specific factors that regulate the timing and initiation of senescence in *B. schlosseri*, and in other ascidians, remain unknown.

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